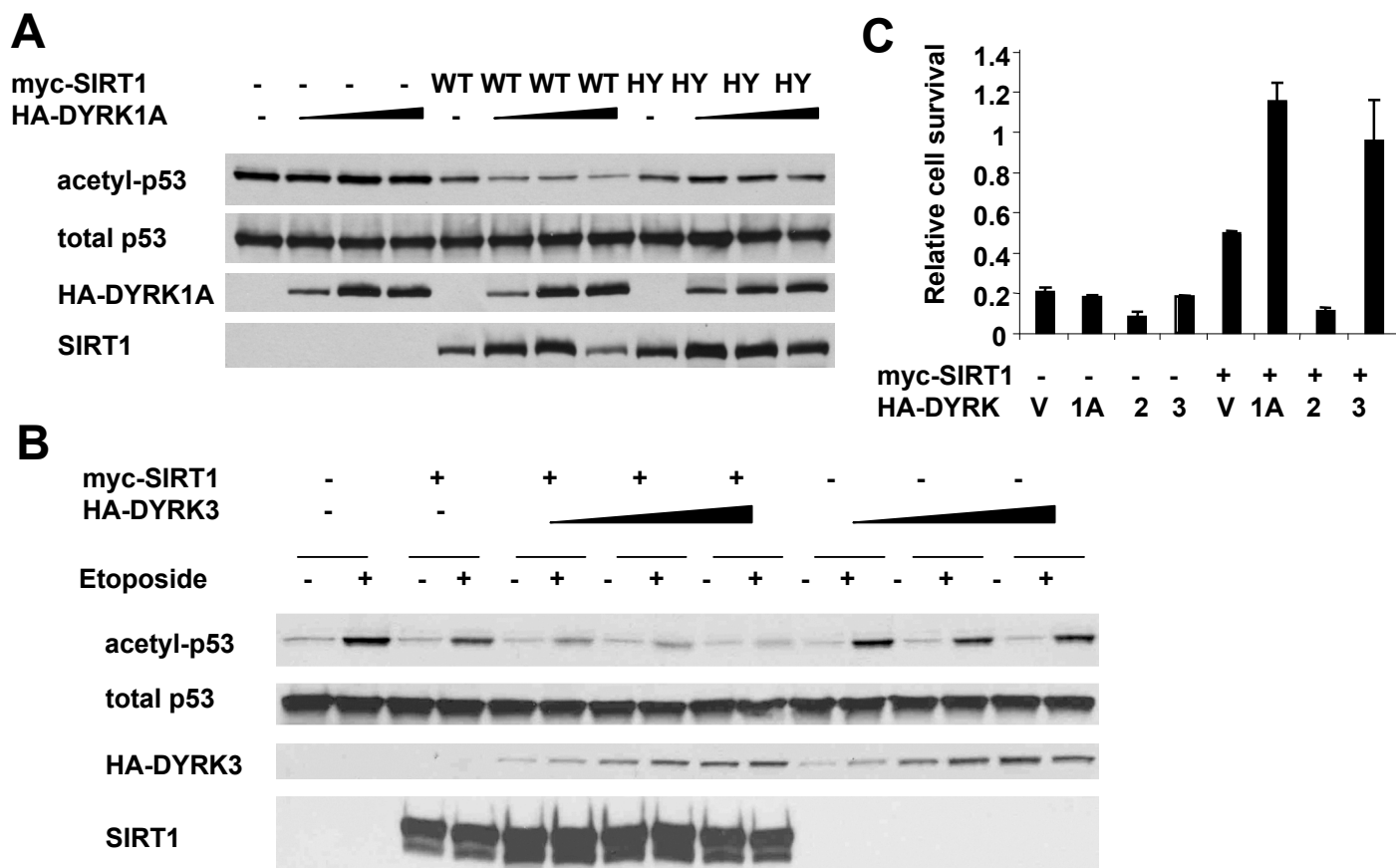
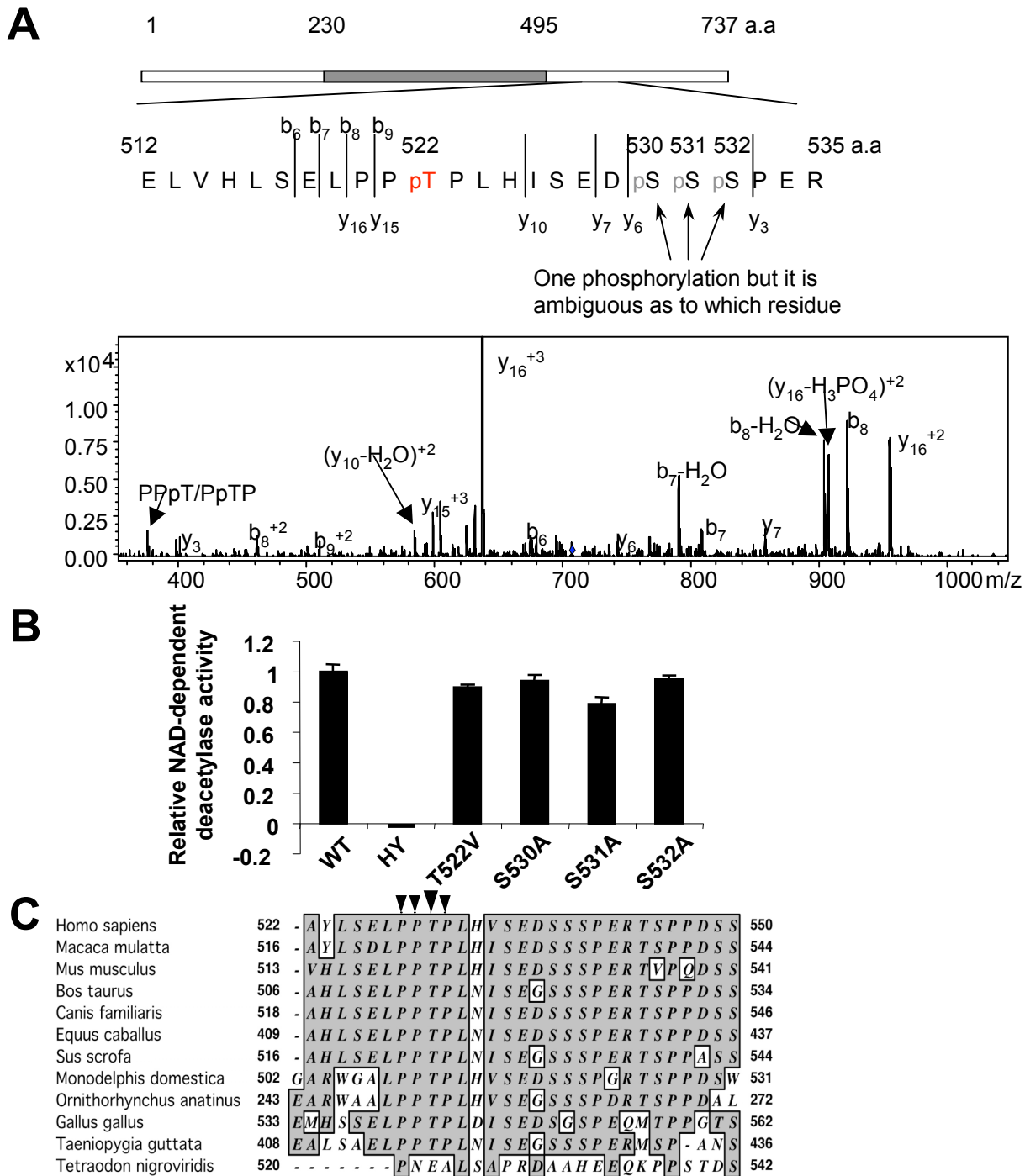


**Fig. S1. SIRT1 interacts with DYRKs.** (A) SIRT1 interacts with DYRK3 in a yeast two-hybrid assay. Yeast cells transformed with indicated plasmids were spotted in a control plate (Sc-L-W) and a selection plates (Sc-L-W-H-A) lacking histidine and adenine. An interaction between GAL4-BD fusion proteins and GAL4-AD fusion proteins is indicated by growth of cells on the selection plate. (B) SIRT1 interacts with DYRKs in U2OS cells. Whole-cell extracts from U2OS cells were immunoprecipitated with control IgG or anti-DYRK antibodies. (C) DYRK1A and DYRK3 interacts with the N- and C-terminal domains of SIRT1. GST-full length SIRT1 or indicated fragments were incubated with HA-tagged DYRK3. Top panel, immunoblot of HA-DYRK3. Bottom panel, ponceau S staining of GST fusion proteins. (D) SIRT1 interacts with the catalytic core domain of DYRK3. GST-full length DYRK3 or indicated fragments were incubated with myc-SIRT1. Top panels, immunoblot of myc-SIRT1. Bottom panel, ponceau S staining of GST fusion proteins.

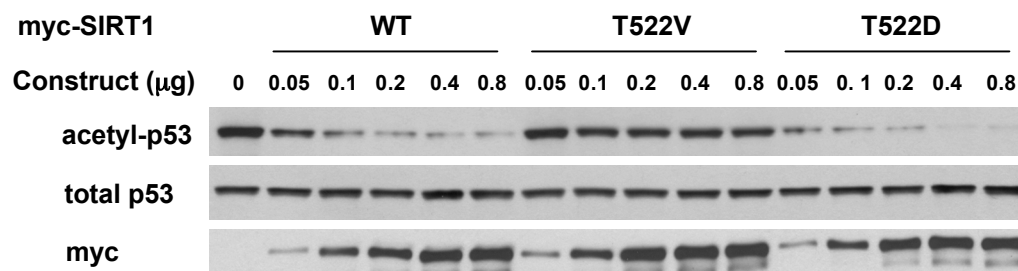


**Fig. S2. DYRK1A and DYRK3 activate SIRT1 deacetylase activity.** (A) DYRK1A dose-dependently promotes SIRT1 deacetylation activity on p53 in HEK293T cells. HEK293T T1RNAi cells were transfected with indicated expressing vectors and cell extracts were analyzed by immuno-blotting with antibodies as shown. (B) DYRK3 dose-dependently promotes SIRT1 deacetylation activity on p53 in HEK293T cells upon genotoxic stress. (C) DYRK1A and DYRK3 promote cell survival through SIRT1 in HEK293T T1RNAi cells.

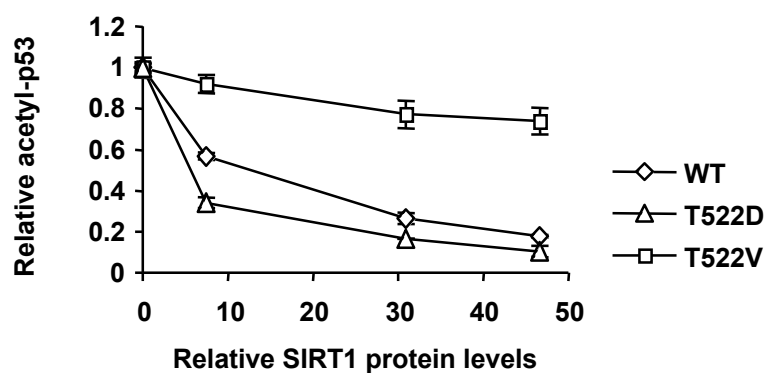


**Fig. S3. DYRK1A and DYRK3 phosphorylate SIRT1 at Thr522.** (A) Mass spectrometry mapping of phosphorylation sites on SIRT1 induced by DYRK3. Myc-SIRT1 was purified from HEK293T T1RNAi cells in the absence or presence of HA-DYRK3. The proteins from the Colloidal Coomassie stained band were digested with trypsin and the resulting peptides were analyzed by LC-MS/MS. (B) Mutations of potential phosphorylation sites on SIRT1 do not affect NAD-dependent deacetylase on a peptide-substrate. Myc-tagged wild-type and mutant SIRT1 were affinity purified from HEK293T T1RNAi cells, and analyzed with *Fluor-de lys* substrate (Biomol). (C) The phosphorylation motif PPTP is conserved in mammalian and avian SIRT1. Indicated SIRT1 proteins were aligned using Macvector protein alignment program using Clustal W method.

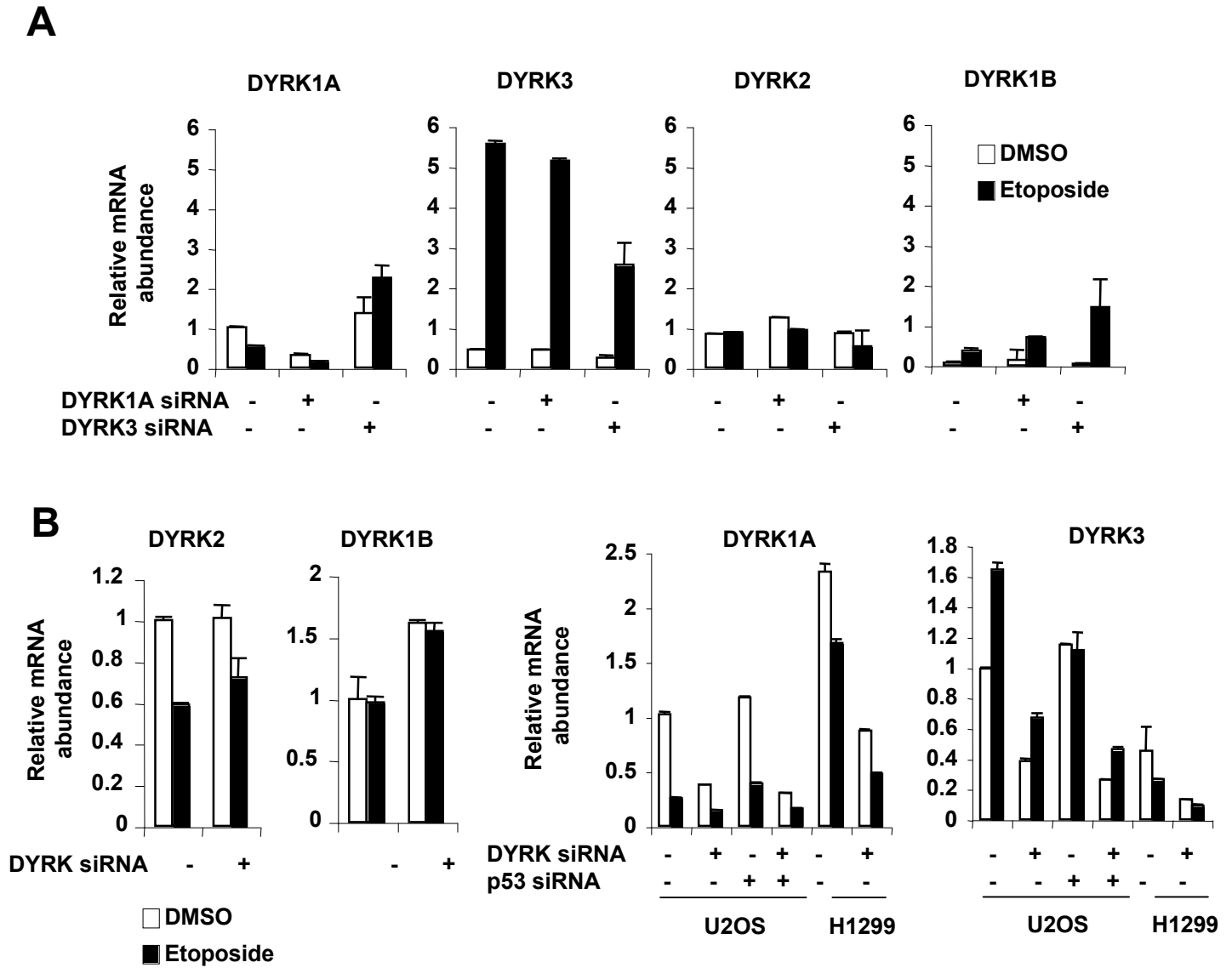
**A**



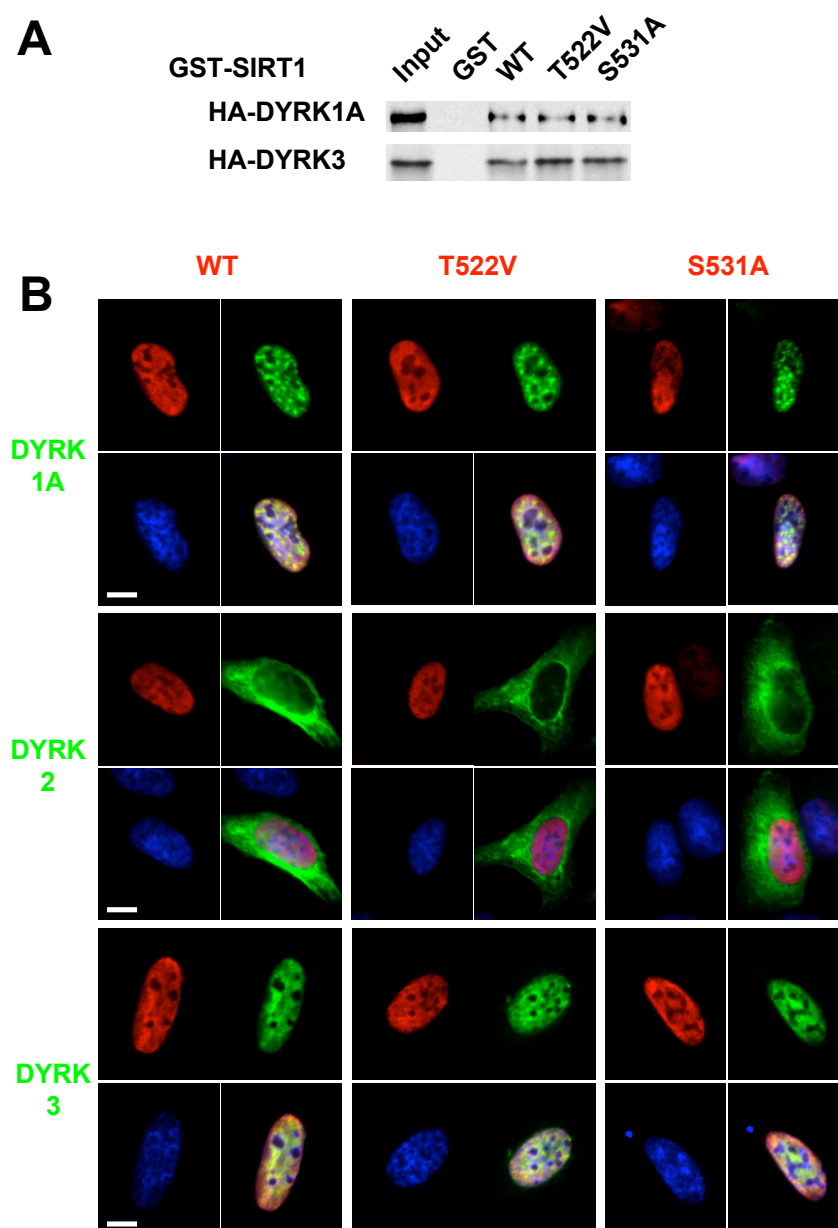
**B**



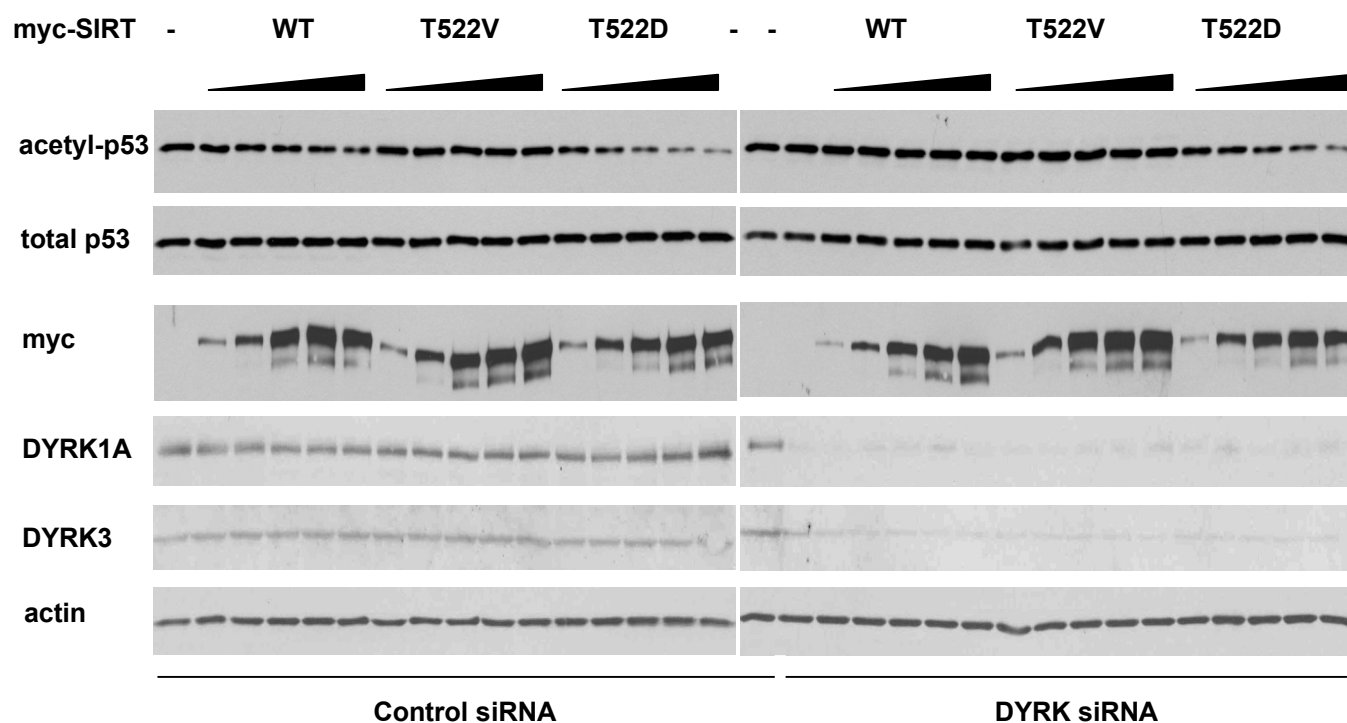
**Fig. S4. The SIRT1 T522D mutant displays increased deacetylase activity *in vivo*.** (A) HEK293T T1RNAi cells were transfected with indicated amount of expression constructs, and were then treated with etoposide and analyzed for the acetylation of p53 as described in the Materials and Methods. (B) the acetylation levels of p53 in (A) were quantified with ImageJ program and normalized to the total p53 levels.



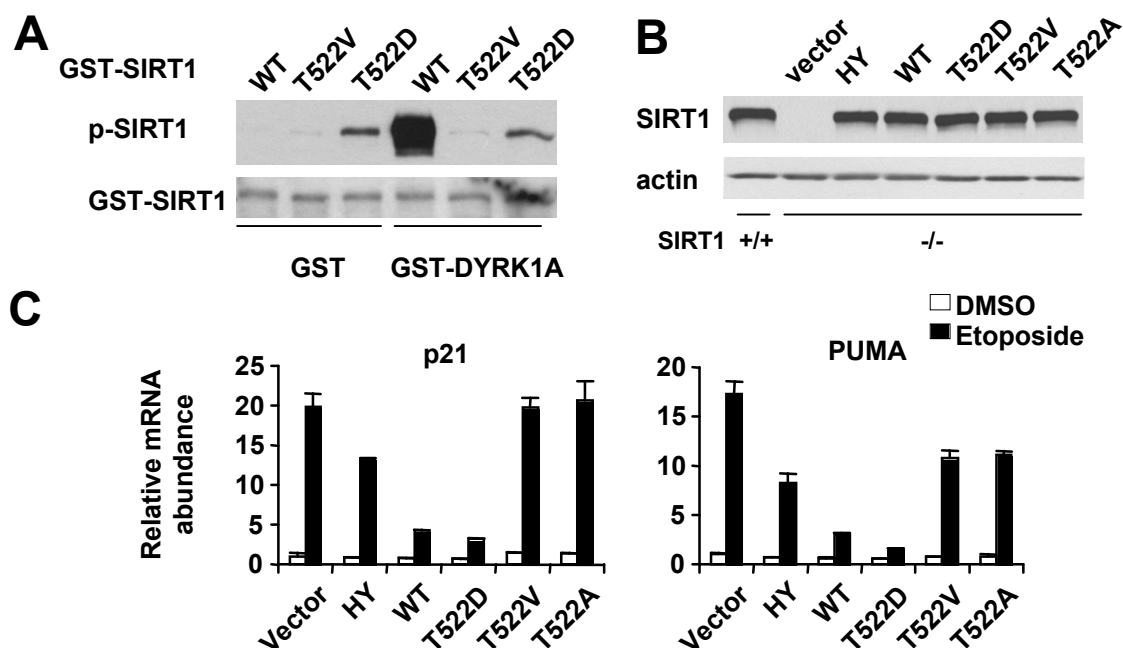
**Fig. S5. siRNA-mediated knockdown of DYRK1A and DYRK3.** (A) Knockdown of DYRK1A and DYRK3 individually results in compensatory increase of other DYRKs. (B) Knockdown of DYRK1A and DYRK3 together in U2OS cells and H1299 cells. Control or siRNA specific to DYRK1A and DYRK3 were transfected into U2OS cells or H1299 cells. 60 hours later, cells were treated with DMSO or Etoposide for 8 h and harvested for mRNA. The expression levels of DYRKs were analyzed by qPCR.



**Fig. S6. The SIRT1 T522V mutant display normal affinity to DYRKs and normal localization.** (A) The SIRT1 T522V mutant interacts with DYRK1A and DYRK3. The interaction between GST-SIRT1 fusion proteins and *in vitro* transcribed and translated HA-DYRK1A or HA-DYRK3 was analyzed by GST pull-down as described in the Materials and methods. (B) The SIRT1 T522V locates in the nucleus. U2OS cells were transfected with indicated constructs, and HA-DYRK (green) and myc-SIRT1 (red) were detected by indirect immuno-fluorescence staining, and the nucleus was stained blue with DAPI. Bar, 10  $\mu$ m.



**Fig. S7. The SIRT1 T522D mutant was constitutively active regardless of cellular DYRK1A and DYRK3 levels.** U2OS cells transfected with control siRNA or siRNA to DYRK1A/3 were transfected with increased doses of vectors (0.01, 0.02, 0.05, 0.1, and 0.2  $\mu$ g) for wild-type SIRT1, T522V, or T522D mutants. Cells were then treated with etoposide and the acetylation of endogenous p53 was analyzed.



**Fig. S8. Phosphorylation of Thr522 is required for the pro-survival activity of SIRT1 in MEFs.** (A) Generation of anti-phospho-T522 antibodies. Rabbit anti-phospho-T522 antisera were generated against the KLH conjugated phosphopeptide KKLSELPP-pT-PLHISE. The antisera detect both *in vitro* phosphorylated wild type SIRT1 and the phospho-mimetic T522D, but not the T522V mutant. (B) SIRT1 protein levels in MEFs. Wild-type (+/+) and SIRT1 deficient (-/-) MEFs infected with retroviruses expressing vector, wild-type, or selected mutant of SIRT1 were analyzed for the protein levels of SIRT1. Note that in SIRT1 deficient MEFs, the expression levels of retrovirus-driven SIRT1 were comparable to those of endogenous SIRT1 in the wild-type MEFs. (C) The SIRT1 T522D mutant displays increased ability to inhibit p53-mediated expression of p21 and PUMA upon genotoxic stress. SIRT1 deficient MEFs expressing indicated SIRT1 protein were treated with DMSO or etoposide for 8 h and mRNA were analyzed by qPCR.